of the produced images makes it easy for the user to distinguish subtle variations in pattern. A directionality score is also calculated based on the peak sharpness in the plot. This new tool was used to compare microtubules in the soleus muscle of wild-type, mdx, utrophin-null and double knockout mice. The directionality scores revealed differences among these mouse lines which could not be appreciated visually and which paralleled the differences observed in EDL muscles.

746-Pos Board B515

Measuring Microtubule Polarity in Spindles with Second-Harmonic-Generation Microscopy

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Microtubule polarity, the fraction of microtubules that point in one direction versus the other, varies throughout spindles. Near the poles, most microtubules point with their plus ends away from the pole, while in the middle of the spindle, equal number of microtubules point in either direction. Antiparallel microtubules in the spindle midzone are thought to be important for determining the localization of proteins that drive spindle elongation in anaphase. While in vitro experiments have suggested mechanisms connecting antiparallel microtubules, protein localization, and spindle elongation, it has been difficult to establish the validity of these models in vivo. A major challenge is that current methods for measuring microtubule polarity, based on electron microscopy, are extremely labor intensive, challenging to combine with protein localization studies, and only provide static snapshots.

Building off of approaches that have been pioneered in neuroscience by Watt Webb's group, we have developed a method using non-linear optical microscopy - second harmonic generation and two-photon fluorescence - to map microtubule polarity throughout spindles. This new technique allows us to quantitatively and nondestructively measure the polarity of microtubules in spindles at high resolution, and study how the distribution of polarity changes over the course of anaphase. We will present a detailed description of the methodology and preliminary results on the temporal evolution of microtubule polarity in the first mitotic spindle of C. elegans.

747-Pos Board B516

Visualization of Individual Cryptophycin-Tubulin Rings by Electron Microscopy Tomography

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The antimitotic drug Cryptophycin-1 (Cr) arrests cell growth by destabilizing microtubules. When added in vitro to either dimeric tubulin or preformed microtubules, Cr induces the formation of single protofilament rings that are highly monodisperse, having a diameter of ~28nm and thickness ~4 nm, and composed of eight tubulin dimers. Here we apply negative stain tomography [Fera, et al, J. Comp. Neurol., 2012] to Cr-tubulin rings. This new technique, which utilizes a beam stable organo-tungsten negative stain, allows construction of tomograms resolving the individual components of the protofilament rings without averaging. Molecular structure of Cr-tubulin rings is revealed in series of en face virtual sections ~4 Å thick positioned parallel to the plane of Cr-tubulin rings. We repeated the observations with rings obtained from tubulin-S, which lack the C-terminal acidic tail filaments that face the center of the Cr-tubulin rings. These tail filaments, normally on the outside of the microtubule and important for MAP and motor protein binding, have a diameter of ~1 nm and significant conformational freedom, making them impossible to detect by averaging methods. Thus, comparison of tomograms from rings of tubulin and tubulin-S will gather further information about these filaments. We also investigate ring-ring interactions in samples of higher concentration to probe their material properties.

748-Pos Board B517

Thermal Noise Imaging Indicates a New Regime of Length-Dependent Persistence Length in Microtubules

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Microtubules perform a diverse set of essential functions within the cell. Most of these functions are mechanical and, therefore, their mechanical properties and the extent to which these properties are tunable are of wide interest. It is known that the persistence length of microtubules depends on factors, such as type and concentration of associated proteins, binding of stabilizers, and their length. We present a new method for measuring microtubule persistence length using a recently developed technique known as three-dimensional thermal noise imaging. Thermal noise imaging is a three-dimensional scanning probe technique capable of probing soft matter at physiological conditions on the nanometer scale. Transverse fluctuations of microtubules have been imaged with thermal noise imaging, and subsequent analysis reveals mechanical properties of the filaments. A novel assay that allows for the isolation of single, grafted microtubules provides a method for measuring the persistence length of microtubules for contour lengths as small as 500 nm. Such a length regime has proven difficult to study using other techniques. We present data that indicates a length regime of microtubules in which the persistence length is significantly smaller than has been measured in longer filaments.

749-Pos Board B518

p150Glued Regulates Microtubule Dynamics in Neurons through Tandem Tubulin-Binding Domains

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Regulation of microtubule dynamics is critical, particularly in neurons, where defects may contribute to neurodegeneration. Here, we demonstrate in vitro using TIRF microscopy and in primary neurons using live-cell imaging that the p150Glued subunit of dynactin regulates microtubule dynamics. To modify dynamics, p150Glued must be dimerized, and it must bind soluble tubulin, an interaction that we find requires tandem CAP-Gly and basic domains. p150Glued is alternatively spliced in vivo, with the full length isoform including both these domains expressed primarily in neurons. Accordingly, depletion of p150Glued in a non-polarized cell line does not alter microtubule dynamics, while p150Glued RNAi in neurons leads to a dramatic increase in microtubule catastrophe. Strikingly, a Parkinson syndrome-associated mutation blocks this microtubule-stabilizing activity both in vitro and in neurons. Together, our data reveal that p150Glued plays a crucial role in promoting microtubule stability in neurons, and that defects in this function may lead to neurodegeneration.

750-Pos Board B519

Obscurin-Dependent Localization of Ankyrin B is required for the Organization of Sub-Sarcolemma Microtubules, Localization of Dystrophin, and Sarcolemmal Integrity in Skeletal Muscle

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Obscurin is a large myofibrillar protein that contains several interacting modules, one of which mediates direct binding to muscle-specific ankyrins. Interaction between obscurin and the muscle-specific ankyrin sAnk1.5 has been found to regulate the organization of the sarcoplasmic reticulum in striated muscles. The organization of dystrophin and β-dystroglycan at costameres is mediated by additional muscle-specific ankyrin isoforms, namely ankB and ankG, localized at the sub-sarcolemma level. In particular, ankB is responsible for the assembly of sub-sarcolemma microtubules required for the correct delivery of dystrophin and β-dystroglycan at costameres. On this basis, we investigated whether obscurin might be involved in the sub-sarcolemma localization of ankB and ankG, and eventually in the assembly of dystrophin and β-dystroglycan at costameres in skeletal muscle fibers. We found that in mice deficient for obscurin, ankB was displaced from its localization at the M-band, while localization of ankG at Z-disk was not affected. In obscurin KO mice, the sub-sarcolemma microtubule cytoskeleton was disrupted and localization at costameres of dystrophin, but not of β-dystroglycan, was markedly reduced. In addition, these mutant mice displayed sarcolemmal fragility and lower forelimbs muscle strength. Altogether, these results support a model where obscurin, by targeting ankB at the M-band, contributes to the organization of sub-sarcolemma microtubules, localization of dystrophin at costameres and to maintenance of sarcolemmal integrity. Accordingly, obscurin appears to represent a multifunctional anchoring protein that on one hand establishes interactions with sarcomeric proteins and on the other hand enables complex formation with extra-sarcomeric proteins, like the muscle-specific ankyrin isoforms, that help to connect the sarcomeres with the SR and with the sub-sarcolemmal cytoskeleton.